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Many breast cancers have elevated levels of the c-Myc oncoprotein. c-Myc associates with transcriptional regulator YY1 and inhibits its ability to modulate transcription. Regulating YY1's activity may be an important facet of c-Myc function. Using co-immunoprecipitations we show that the amount of YY1 associated with c-Myc is dependent upon c-Myc levels, consistent with the notion that c-Myc modulates YY1 activity. The region of YY1 required for association has been mapped and association-defective mutants are under study. Another study is underway to determine how normal and mutant YY1 transgenes affect cell growth and development *in vivo*.

We have identified the zinc finger protein Blimp-1 as a repressor of c-*myc* transcription. Ectopic expression of Blimp-1 can decrease c-Myc levels and cause preB cells to undergo apoptosis. As a repressor of c-*myc* expression, Blimp-1 might function as a tumor suppressor. Blimp-1 maps to human chromosome 6q22.1-3--a region sometimes deleted in primary breast cancer, ovarian cancer and non-Hodgkins lymphoma. We will pursue the exciting possibility that Blimp-1 may be inactivated by deletion and mutation in some breast and other cancers.

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Kathryn Calame 10/28/96

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KATHRYN L. CALAME, DAMD17-94-J-4102

(For clarity, two individual introduction, methods, results and discussion sections are presented. This is followed by one reference and one figure legend section for the entire report.)

## I. THE ROLE OF C-MYC IN MODULATING YY1 ACTIVITY

**Jin Yu, Ajay Shrivastava and Kathryn Calame**

### INTRODUCTION

The c-*myc* proto-oncogene encodes a ubiquitously expressed nuclear phosphoprotein (1-3). Despite clear evidence that c-Myc is important in the control of cellular proliferation, differentiation, apoptosis and transformation (1), the molecular mechanisms by which c-Myc functions are not completely understood.

c-Myc has DNA-binding, dimerization and transactivations domains common to other transcriptional activators (1). c-Myc/Max heterodimers can activate the p53 (4), ECA39 (5), α-prothymosin (6), DHFR (7), ornithine decarboxylase (8, 9) and cdc25 (10) genes However, the number of known c-Myc/Max-regulated genes remains small, suggesting that important facets of c-Myc action remain uncharacterized. In addition, some mutants of c-Myc are defective in transformation ability but not in transcriptional activation ability (11), suggesting that functions other than transcriptional activation may be important for c-Myc function.

c-Myc can also suppress the expression of specific genes including the major histocompatibility complex class I antigens HLA-A2 (12) and HLA-C (13), cyclin D1 (14), integrin LFA-1 (15), adhesion receptor N-CAM (16) and transcription factor C/EBPα (17). c-Myc also represses its own transcription (18). No c-Myc/Max binding sites have been identified in the regulatory regions of these genes and the mechanism(s) by which Myc suppresses their transcription is poorly understood although for the C/EBPα and albumin genes c-Myc appears to act on initiator elements (11).

We have previously shown that c-Myc can physically associate *in vitro* and in the yeast two-hybrid system with transcription protein YY1 (19). YY1 is a ubiquitiously expressed zinc finger protein (20-24) which functions as a transcriptional repressor, activator or initiator, depending upon the context of its binding site. YY1 binding sites are widely distributed in many cellular and viral

promoters (25). Association with c-Myc inhibits the transcriptional activating and repressing abilities of YY1 (19). We have previously suggested that c-Myc may regulate transcription of YY1-dependent genes by modulating YY1 activity (19). Since regulation of YY1 activity could provide an additional mechanism for c-Myc-dependent transcriptional regulation, we are investigating the physiological relevance of this association.

## METHODS

**Antiserum preparation.** Murine c-Myc antiserum was generated by injecting bovine albumin serum (BSA) coupled to a synthetic peptide representing the C-terminal 13 amino acids of murine c-Myc into rabbits.

**Co-immunoprecipitation.** $2x10^7$ NIH 3T3 cells were washed, resuspended in buffer X (50 mM Tris pH 7.5, 50 mM NaCl, 7 mM CaCl2, 10 mM EDTA, 5 mM DTT, 0.5% NP40, 1 mM PMSF, 20 mg/ml of pepstatin, leupeptin and aprotinin), sonicated on ice and centrifuged for 30 minutes at 13000 rpm to obtain lysates used for coimmunoprecipitation. Lysate was precleared by incubating 20 minutes with protein A-sepharose beads in buffer X. Lysate was kept on ice for 1 hr, incubated α–Myc antisera for 2 hrs, and then protein A-sepharose beads for 4 hrs. Proteins bound to beads were resolved by SDS-PAGE and visualized by immunoblotting with α-Myc polyclonal antiserum and α-YY1 monoclonal antibody. One tenth of each immunoprecipitate was used for the c-Myc blots and 9/10 for the YY1 blots.

**GST assays** GST assays were performed as described (19). Association assays were done in buffer that had final conditions of 50 mM NaCl, 7 mM CaCl2, 10 mg/ml BSA, 5 mM DTT, 1 mM PMSF, 20 mg/ml of aprotinin, leupeptin and pepstatin.

## RESULTS

**The Amount of YY1 Associated with c-Myc Varies When c-Myc Levels Change.**

We reasoned that changes in c-Myc protein levels might regulate the ratio of free YY1 to c-Myc-associated YY1 in situations where overall YY1 levels remained unchanged. To test this hypothesis we coimmunoprecipitated YY1 associated with c-Myc from 3T3 cells in which c-Myc is induced in response to serum stimulation (26, 27) and from murine erythroleukemia (MEL) cells where c-Myc levels decrease upon differentiation in response to DMSO (28). c-Myc, YY1 and YY1 associated with c-Myc were determined before and after the treatments to alter c-Myc levels. Fig. 1A shows that when quiescent 3T3 cells were treated with serum for two hours, c-Myc was strongly induced but YY1 levels did not change during this time (Fig. 1B).

Measured by co-immunoprecipitation, the amount of YY1 associated with c-Myc also increased from undetectable to detectable amounts (Fig. 1C). Thus mitogenic stimulation induces c-Myc, resulting in association between YY1 and c-Myc and thus decreasing the amount of free YY1 available to regulate YY1-dependent genes. Similarly, when MEL cells were stimulated to differentiate in response to DMSO, c-Myc levels decreased 75% and YY1 associated with c-Myc decreased 62% although YY1 levels did not change (data not shown). These results support a model in which changes in c-Myc levels modulate the availability of active YY1.

**The region of YY1 which is necessary to bind c-Myc is located between amino acids 144 and 208.**

We wished to determine the region of YY1 which is required to associate with c-Myc. This information will help us understand the nature of the protein-protein interaction and will also allow specific YY1 mutants, which are defective for association with c-Myc, to be tested functionally in a number of assays. A series of plasmids encoding C-terminal and N-terminal truncations of YY1 were generated and tested for their ability to associate with c-Myc in the GST binding assay. As shown in Fig. 2, C-terminal truncations up to amino acid 208 were capable of associating with c-Myc but truncation to amino acid 201 ablated binding. N-terminal truncations to amino acid 116 were able to bind but truncation to amino acid 144 ablated binding. Therefore, the region required for binding to c-Myc is contained within amino acids 144-208. Further, it appears that amino acids 201-208 are required. Therefore we are currently engineering a small deletion in YY1 encompassing amino acids 201-208. This mutant, Δ201-208 YY1, will be tested for its ability to repress and activate transcription and for its ability to be inhibited by c-Myc.

**YY1 transgenic mice.**

If part of c-Myc's biological activity is due to its ability to inhibit YY1 activity, one would predict that ectopic expression of YY1 might have effects similar to partial repression of c-*myc*. This might include slow growth of particular cell lineages or failure of certain cells to undergo apoptosis. In addition, ectopic expression of YY1 is likely to have additional effects which are not usually or ever affected by c-Myc levels. To address the importance of c-Myc in regulating YY1 as well as the overall importance of YY1, we wish to compare the effect of wt YY1 and Δ201-208 YY1 when expressed as transgenes in mice. Since a completely c-Myc

deficient mouse dies during embryonic development, our strategy is to target transgene expression to particular tissues which are dispensable for mouse development. Since lymphoid cells fulfill this criterion, in our initial experiments YY1 transgene expression will be targeted to B and T lymphocytes by using the IgH Eμ enhancer. These experiments are underway and wt YY1 transgenic animals have been made. We have 15 founders in which transgene copy number varies from 1 to >10 (Table I). They are being bred and the number and developmental pattern of their B and T cells will be analyzed using flow cytometry. As soon as we confirm that Δ201-208 YY1 retains its ability to modulate transcription but fails to associate with c-Myc, transgenic mice expressing this protein from the same regulatory elements will be made and analyzed.

## DISCUSSION

In addition to YY1, c-Myc also associates with Max, TBP (29, 30), p107 (31), TFII-I(32) Rb (33) and TFII-B (A. Berrier and K. Calame, unpublished). In addition to c-Myc, YY1 associates with TBP, TFII-B, Sp1 (34, 35), nucleolar protein B23 (36), p300 (37) and transcription factor TFE3 (A. Shrivastava and K. Calame, unpublished); YY1 has also been identified as a nuclear matrix-associated protein (38). In spite of the complicated possible associations in the nucleus, we have shown that physiological changes in c-Myc are sufficient to alter the amount of YY1 associated with c-Myc. Thus, changes in c-Myc levels could alter the amount of YY1 enough to change expression of YY1-dependent genes. The growing list of YY1-dependent genes currently includes 17 cellular genes and 8 viral genes, including ubiquitously expressed genes (22, 39-42), tissue specific genes (23, 43-46) and proto-oncogenes c-*fos* (47) and c-*myc* (48, 49). YY1 binding sites are also found in proto-oncogene N-ras and the cell cycle regulated E2F1 promoter (50, 51). Altered expression of YY1-dependent genes could have important effects on cell growth and, thus, could be responsible for some effects of c-Myc.

The most dramatic changes in c-Myc levels are associated with tumors where c-*myc* gene expression is deregulated due to chromosomal translocation, gene amplification or retroviral insertion (52-55). The aberrantly high levels of c-Myc in tumors is likely to cause a significant change in expression of YY1-dependent genes. It will be interesting to determine if any genes which are differentially expressed in tumors with elevated levels of c-Myc lack c-Myc/Max sites and are YY1-dependent.

## II. BLIMP-1 IS A REPRESSOR OF C-MYC TRANSCRIPTION AND A POSSIBLE TUMOR SUPPRESSOR IN SEVERAL HUMAN CANCERS

**Yi Lin, Jin Yu, Kwok-kin Wong and Kathryn Calame**

### INTRODUCTION

c-Myc functions at a critical decision point of cell growth to favor proliferation and to block terminal differentiation (1, 56). c-Myc is present in dividing cells but is not expressed in quiescent or terminally differentiated cells (55) In fact, addition of exogenous c-Myc blocks terminal differentiation of several hematopoietic cell lines (28, 57-64) and of myogenic cells (65, 66) while inhibitors of c-Myc expression accelerate terminal differentiation of promonocytic HL60 cells (67-69), M1 leukemic myeloid cells (70), F9 teratocarcinoma cells (71) and human esophagael cancer cells (72).

We previously identified a plasmacytoma-specific protein, Plasmacytoma Repressor Factor (PRF), which binds in the *c-myc* promoter 290 bp 5' of the P1 transcriptional start site. PRF represses *c-myc* transcription in plasmacytomas (73, 74). The PRF site in the c-*myc* promoter is similar to interferon stimulated response elements (ISREs) and to the positive regulatory domain 1 (PRD1) in the β-interferon promoter. PRD1-BF1 is a zinc finger protein which was cloned by virtue of its ability to bind the PRD1 site; PRD1-BF1 inhibits transcription of the β–IFN promoter (75). Recently the murine homologue of PRD1-BF1, Blimp-1, was identified as a protein which is induced upon stimulation of the $BCL_1$ B-cell lymphoma line with IL-2 + IL-5 (76). Ectopic expression of Blimp-1 can drive B-cell terminal differentiation and Blimp-1 is expressed only in plasmacytomas and mature B cells; however its mechanism of action is not well-understood (76).

*In vitro* binding studies using recombinant Blimp-1 and endogenous PRF, showed that they bound the same site in the c-*myc* promoter and that antiserum to Blimp-1 ablated endogenous PRF binding. Furthermore, in a cotransfection assay in pre-B cells, Blimp-1 repressed the c-*myc* promoter in a manner which was dependent upon the PRF site. Thus, it appears that Blimp-1 is a repressor of c-*myc* transcription in B cells. We wish to study further the relationship between Blimp-1, c-*myc* transcription and cell growth phenotype. Our initial studies have focused on the activity of Blimp-1 in B cells because of its previously demonstrated importance

in terminal B-cell differentiation. These studies provide a starting point for future studies in which the role of Blimp-1 will be studied in other terminally differentiated cells and in tumor cells

## METHODS

**$BCL_1$ cell differentiation and analysis.** $BCL_1$ cells in culture were treated with IL2 + IL5 and whole cell extracts were prepared at various time. Ten μg of each sample was electrophoresed on SDS-polyacrylamide gels (8%), transferred to a nitrocellulose membrane and immunoblotted with polyclonal antiserum raised against the C-terminus of murine c-Myc. The bands were quantitated and the amounts relative to that prior to treatment (0 hr.) were calculated. RNA was also prepared and analyzed by Northern blotting using a *blimp-1* cDNA probe (76) and a control *β-actin* probe.

**Ectopic expression of Blimp-1 in 18-81 cells.** A pBJ-neo plasmid containing either antisense (Blimp AS) or sense (Blimp S) *blimp-1* cDNA was cotransfected into 18-81 pre-B cells with the $pSV_2$ c-Myc expression vector or a $pSV_2$ control vector. Cells were diluted into 96 well plates, cultured with 800 mg/ml G418 and resistant colonies were counted 10 days later. For inducible expression, the *blimp-1* cDNA was cloned into a plasmid under the control of the sheep metallothionein promoter (77) and stably transfected into 18-81 pre-B cells using selection in G418.

## RESULTS

**Endogenous c-Myc is downregulated upon induction of Blimp-1 in BCL1 cells.**

$BCL_1$ is a mature B-cell line which, upon stimulation with IL2 + IL5, differentiates into a plasma cell-like state accompanied by Ig secretion and morphological changes (78, 79). Blimp-1 is induced early in $BCL_1$ differentiaion (76). Based on the ability of transfected Blimp-1 to repress the *c-myc* promoter, we predicted that differentiation-dependent induction of Blimp-1 in $BCL_1$ cells would cause a decrease in endogenous c-Myc. Seventy-two hours after $BCL_1$ cells were treated with IL2 + IL5, differentiation was verified by increased immunoglobulin secretion and changes in cell size as indicated by changes in forward vs. orthogonal scatter (not shown). c-Myc levels during this period were assessed by immunoblot (Fig. 3A). Following a transient increase, cMyc levels decreased approximately four fold between 1 and 2 hrs of IL2 + IL5 stimulation and remained low for 24 hours. Northern analyses showed that 1 hour after stimulation, *Blimp-1* mRNA increased

approximately 5 fold, as previously reported (76) (Fig. 3B). These data are consistent with the notion that Blimp-1 represses endogenous *c-myc* transcription, leading to decreased c-Myc protein levels.

**Constitutive expression of Blimp-1 suppresses growth of 18-81 cells and this effect is overcome by expression of c-Myc.**

Since Blimp-1 appears to be a master regulator which drives terminal B-cell differentiation, it is reasonable that the *c-myc* gene would be an important target for Blimp-1-mediated repression. To obtain more direct evidence for this idea, we tried to stably transfect pre-B cells with a Blimp-1 expression plasmid containing the $neo^R$ gene. However, few G418-resistant clones were obtained, demonstrating that over-expression of Blimp-1 suppressed cell growth as measured by $neo^R$ colony formation (not shown). This is consistent with the findings of others who have also experienced difficulty in obtaining Blimp-1 over-expressing cell lines (76) . Since Blimp-1 can repress *c-myc* transcription, we speculated that Blimp-1-dependent suppression of growth might be caused directly by the decreased abundance of c-Myc. Therefore, we tested whether ectopic expression of c-Myc could reverse Blimp-1-dependent growth suppression. 1881 cells were transfected with combinations of the pBJ1-neo Blimp-1 expression plasmid or the same plasmid with Blimp-1 in antisense orientation and the $pSV_2$ *c-myc* expression plasmid or a control pSV2 plasmid. After 10 days of culture, the number of G418 resistant colonies was determined (Fig. 4). The Blimp-1 antisense plasmid provided a control to establish transfection efficiency and functionality of the $neo^R$ gene. As before, few colonies were obtained with the Blimp-1 expression plasmid. Upon immunoblot analysis using antiserum to Blimp-1, 16/16 clones failed to express Blimp-1 (not shown). However, co-expression of c-Myc partially reversed Blimp-1 suppression of growth. Compared to control antisense Blimp-1 and *c-myc* plasmids, transfections with Blimp-1 sense and *c-myc* plasmids gave 17-65% the number of control colonies, depending on the ratio of *c-myc* to Blimp-1 plasmid transfected (Fig. 4B). Northern blot analyses confirmed that, unlike the colonies from transfection with the Blimp-1 expression plasmid alone, 4/5 of these colonies expressed Blimp-1 (not shown). This demostrates that ectopic c-Myc blocks the growth suppressing effect of high Blimp-1 expression. The simplest interpretation of these results is that repression of

*c-myc* transcription by over-expressed Blimp-1 is directly responsible for the failure of Blimp-1 over-expressing cells to grow.

**Induction of ectopic Blimp-1 in 18-81 cells leads to apoptosis.**

We wished to test more directly the ability of Blimp-1 to repress transcription of the endogenous c-*myc* gene in 18-81 cells and to learn how ectopic expression of Blimp-1 caused the cells to stop growing. Therefore, 18-81 cells were stably transfected with an inducible Blimp-1 expression plasmid in which the expression of Blimp-1 was dependent upon the sheep metallothionein promoter (MT) and could be regulated by cadmium in the culture medium. The analyses of these transfected clones has begun but is not yet complete. Preliminary Northern analyses confirm that treatment with increasing concentrations of Cd results in increased Blimp-1 mRNA and immunoblots show that c-Myc levels decrease significantly following Cd treatment (data not shown). Growth of cells harboring MT-Blimp-1 is slow compared to MT control cells when 20 μM Cd is present (Fig. 5). In addition, when a the concentration of Cd is varied, cells harboring MT-Blimp-1 begin to die in 10 μM and are >90% dead in 40 μM Cd. Analysis of genomic DNA from MT-Blimp-1 cells in the presence and absence of Cd also reveals that the dying cells are undergoing apoptosis as evidenced by characteristic DNA fragmentation (Fig. 5). More complete analyses of these cells is in progress to confirm that induced Blimp-1 represses c-*myc* expression and to document more completely the apoptotic death of Blimp-1 expressing cells. We tentatively conclude from these results that in the 18-81 cell model, Blimp-1-dependent reduction of c-Myc causes programmed cell death.

**Blimp-1 activity may involve association with YY1.**

In the c-*myc* (48, 49) and β-casein (44)gene promoters, there are YY1 sites which are located near apparent Blimp-1 sites. Interestingly, mammary-specific expression of the β-casein promoter in response to prolactin appears to proceed by induction of Stat 5 which competes away binding of the YY1 repressor (80, 81). Since Blimp-1 expression correlates with terminal differentiation in B cells, we hypothesize that it may also be expressed in other terminally differentiated cells, including mammary gland cells. It is possible that in one or both of the promoters where their binding sites are located near each other, YY1 and Blimp-1 may interact physically and/or functionally. To address this question, we have performed GST assays to ask if the two proteins associate *in vitro*. Our results show that that

the two proteins do associate *in vitro* (data not shown). We are in the process of determining if this association affects binding of either protein to the c-*myc* and β-casein promoters and if it affects the function of the proteins in these two contexts. We are also studying the expression pattern of Blimp-1 to determine if it is expressed in mammary cells.

**Blimp-1 may be a tumor suppressor in breast cancer and other cancers.**

Since Blimp-1 is a repressor of c-*myc* transcription, one might predict that in some cell contexts, Blimp-1 would act as a tumor suppressor. Therefore, we wished to determine if the chromosomal location of the human *blimp-1* gene correlated with any known or potential tumor suppressor loci. We have determined, by taking advantage of data from the human genome project, that Blimp-1 maps to chromosome 6q 22.1-22.3. Interestingly, there are several reports that deletions in this region of 6q occur in primary breast cancer (82, 83), in ovarian cancer (84) and in non-Hodgkins lymphomas (85-87). This is quite intriguing and prompts us to study further the status of *blimp-1* in these tumors. We are currently trying to identify highly polymorphic RFLPs from the human *blimp-1* gene so that DNA from primary breast tumors and NHLs can be analyzed by Southern blotting to determine if loss of heterozygosity in *blimp-1* correlates with disease. If so, further analyses of *blimp-1* in the tumors will be performed.

## DISCUSSION

Our data have convincingly shown that Blimp-1 is a repressor of c-*myc* transcription in B cells and that Blimp-1 is the protein which we previously identified as Plasmacytoma Repressor Factor. This conclusion is based on several types of evidence. 1) Recombinant Blimp-1 and endogenous PRF both bind the PRF site in the c-*myc* promoter and give rise to complexes with identical electrophoretic mobility. 2) The endogenous PRF-DNA complex is ablated with antiserum against Blimp-1. 3) Blimp-1 represses the c-*myc* promoter in a manner which is dependent on the PRF site in a cotransfection assay. 4) When BCL1 cells are induced to differentiate, endogenous Blimp-1 increases and endogenous c-Myc decreases. It may be, in fact, that repression of c-*myc* transcription is sufficient to drive terminal differentiation of certain B cells. This hypothesis is testable in the BCL1 model and such experiments are underway.

The tissue specificity of Blimp-1's ability to repress c-*myc* transcription, however, remains unclear. It appears that Blimp-1 may not repress c-*myc* in fibroblasts, based on our cotransfection experiments and on the fact that 3T3 transfectants which express ectopic Blimp-1 grow normally. However, it is important to learn whether Blimp-1 is expressed and whether it can repress c-*myc* transcription in other tissues, especially mammary cells. It is our hypothesis that Blimp-1 expression and repression of c-*myc* transcription is unlikely to be limited to B-cells and experiments are underway to test this hypothesis.

Finally, the intriguing discovery that Blimp-1 maps to a region of chromosome 6q which has previously been suggested to encode a tumor suppressor is exciting and potentially significant. It is particularly interesting that there are reports that deletions of 6q occur in some primary breast cancers. Thus, it is very important for us to determine the expression pattern and activity of Blimp-1 in mammary tissue. It is also important for us to determine the status of the *blimp-1* gene in tumors which harbor deletions of 6q. If there is loss of heterozygosity, we will determine if the remaining *blimp-1* gene is mutated and if ectopic expression of Blimp-1 alters the transformed phenotype of tumor cells lines which harbor 6q deletions. These experiments are underway at present.

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**TABLE I**

**Eμ-YY1 Transgenic Founders**

| Mouse # | Transgene Copy Number* |
|---|---|
| 30 | 10 |
| 34 | 9 |
| 35 | >10 |
| 43 | 1 |
| 50 | 4 |
| 51 | 1 |
| 52 | >10 |
| 4 | 6 |
| 5 | 2 |
| 6 | 1 |
| 9 | 1 |
| 18 | 10 |
| 22 | 4 |
| 26 | 4 |
| 31 | 4 |

Tail DNA from each founder was anaylzed by Southern blotting using a YY1 cDNA probe. Copy number was determined by phosphoImager quantitation and comparison between the single copy endogenous gene and the transgene.

## FIGURE LEGENDS

**Figure 1. YY1/c-Myc complexes increase when 3T3 cells are stimulated with serum.** A) Immunoblot developed with α-c-Myc antiserum. Lane 1, lysate from serum stimulated cells; lanes 2-3, immunoprecipitates using α-c-Myc antiserum from serum starved cells; lanes 3-4 immunoprecipitates using α-c-Myc antiserum from cells 2 hrs. after adding serum; lanes 6-7 immunoprecipitates from serum-treated cells using pre-immune serum. B) Immunoblot of cell lysates before and after serum stimulation developed with α-YY1 antiserum. C) Immunoblot developed with α-YY1 antiserum. Lanes are identical to those in A.

**Figure 2. Mapping the YY1 region required for association with c-Myc.** A) A diagram of YY1 protein. The C-terminal and N-terminal truncations which were expressed as fusion proteins with GST are listed and their ability to associate with c-Myc is indicated in the right column. The region found to be necessary for association with c-Myc is indicated by a bar. B) Association of various GST fusion proteins with $^{35}S$ labeled c-Myc. All GST fusion proteins were present at similar levels as judged by Coomassie stained SDS-PAGE (data not shown)

**Figure 3. Kinetics of Blimp-1 induction and c-Myc reduction during $BCL_1$ cell differentiation.** (A) $BCL_1$ cells were treated with IL2 + IL5 and whole cell extracts were prepared at various time. Ten μg of each sample was electrophoresed on SDS-polyacrylamide gels (8%), transferred to a nitrocellulose membrane and immunoblotted with polyclonal antiserum raised against the C-terminus of murine c-Myc. The bands were quantitated and the amounts relative to that prior to treatment (0 hr.) are shown below the lanes. (B) RNA was also prepared and analyzed by Northern blotting using a *blimp-1* cDNA probe (31) and a control *β-actin* probe. The relative amounts of *blimp-1* mRNA are given below the lanes.

**Figure 4. c-Myc blocks the growth suppression effect of high Blimp-1 expression.** (A) A pBJ-neo plasmid containing either antisense (Blimp AS) or sense (Blimp S) *blimp-1* cDNA was cotransfected into 18-81 pre-B cells with the $pSV_2$ c-Myc expression vector or a $pSV_2$ control vector. The amount (μg) of each plasmid used is shown on the left. Cells were diluted into 96 well plates, cultured with 800 mg/ml G418 and resistant colonies were counted 10 days later. Results show the average of 3 independent experiments. (B) A graphic representation of the data in A. "0" for Blimp-1 indicates 5 μg antisense Blimp-1 plasmid and "0" for c-Myc indicates 10 μg

pSV2. For each transfection with the Blimp-1 plasmid, the corresponding transfection with antisense Blimp-1 was taken as 100%.

**Figure 5. Induction of ectopic Blimp-1 leads to apoptosis of 18-81 cells.** Blimp-1 under the control of a sheep MT promoter was stably transfected into 18-81 cells. A) Clones harboring MT-Blimp-1 or a control MT plasmid were cultured in 20 μM Cd to induce Blimp-1 expression. Closed circles show viable cells and open circles show % viable cells. B) Representative clone harboring MT-Blimp-1 was cultured for 36 hours in different concentrations of Cd. Closed circles show viable cells and open circles show % viable cells. C) Ethidium bromide stained gel showing electrophoresis pattern of genomic DNA isolated from cells in 20 μM Cd (Cd) or without Cd (0). The first two lanes show DNA from a MT control and the following lanes show DNA from 2 MT-Blimp-1 clones. Triangle indicates increasing time in Cd.

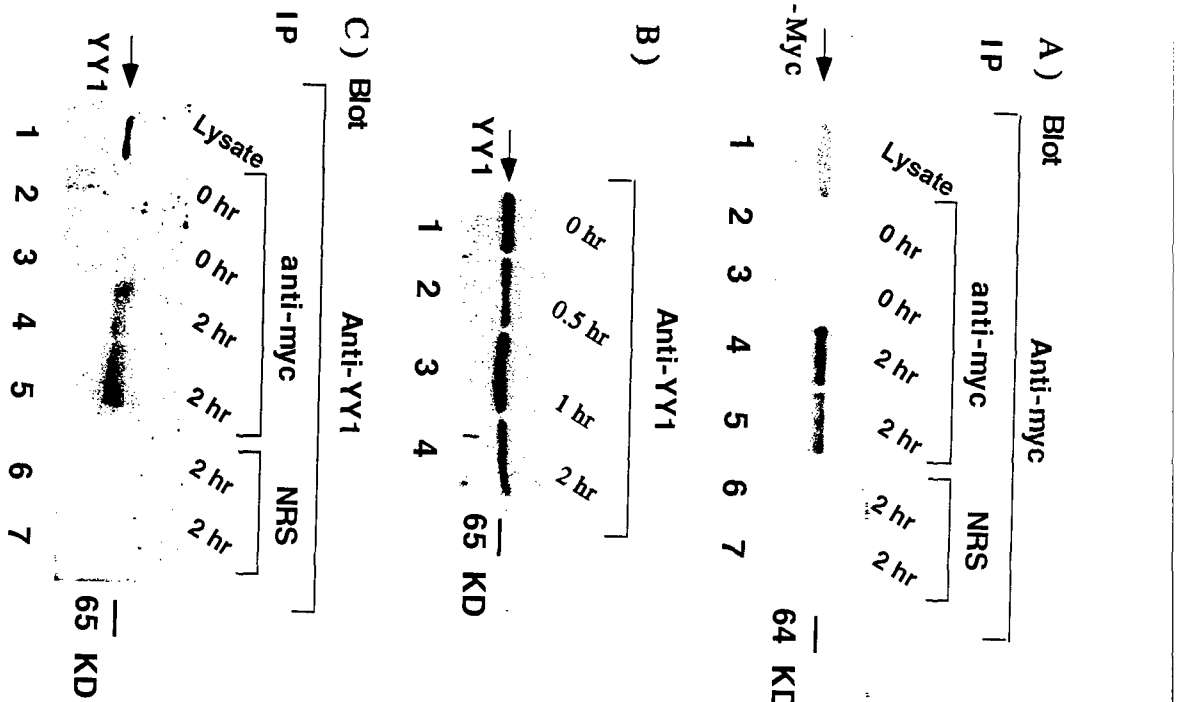
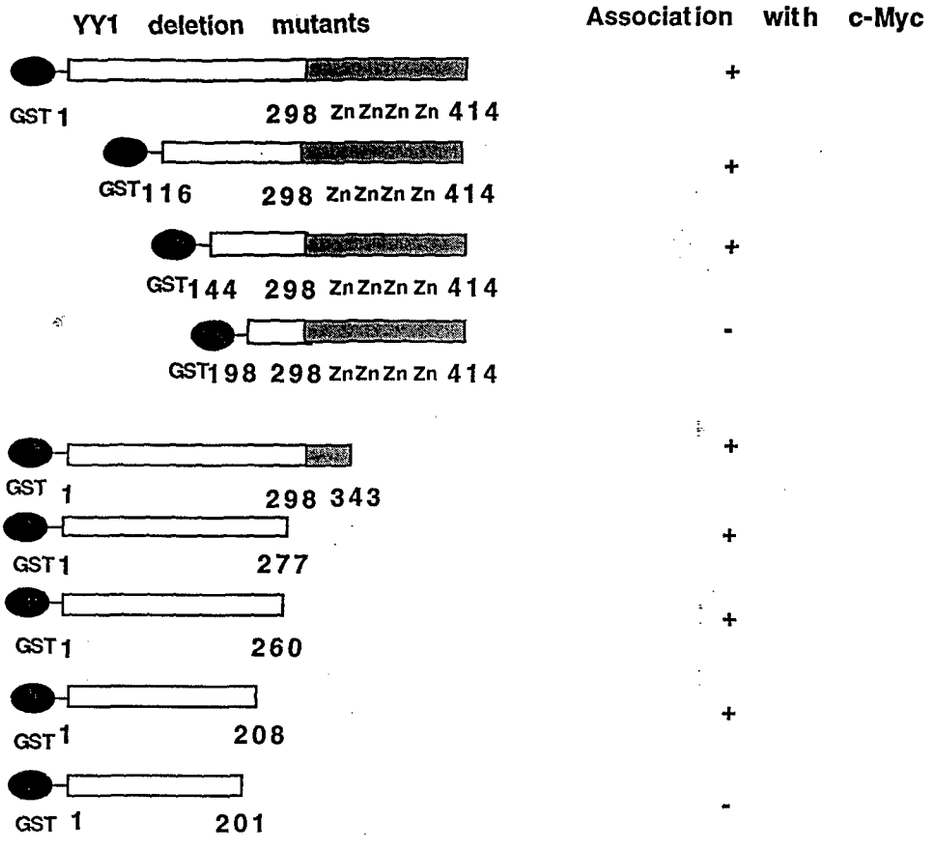
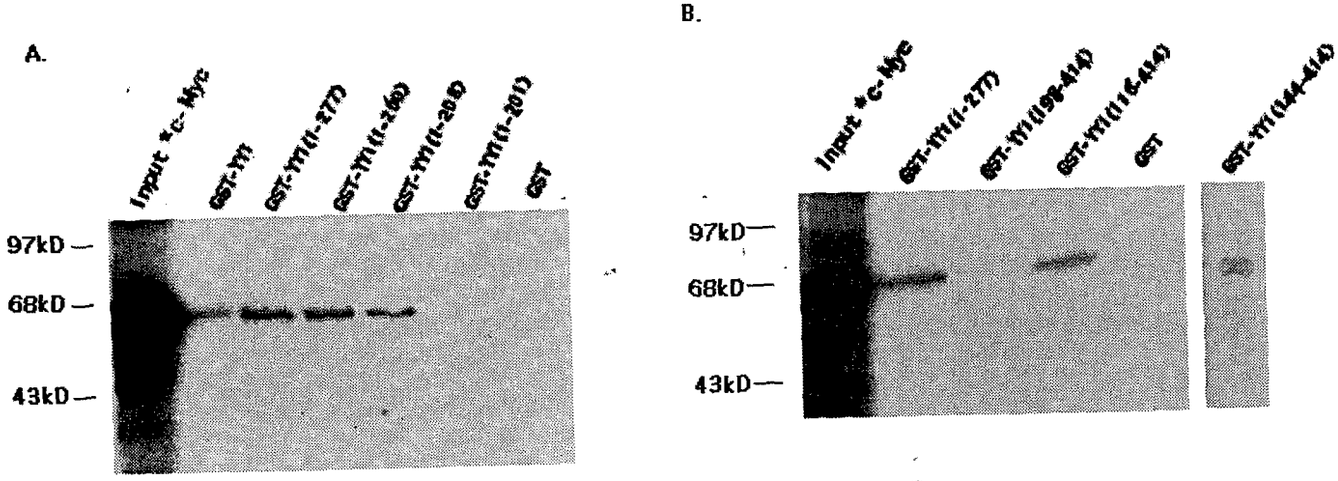

A) Blot
IP
Anti-myc
Lysate
anti-myc
NRS
0 hr
0 hr
2 hr
2 hr
2 hr
2 hr
c-Myc
64 KD
1 2 3 4 5 6 7
B)
Anti-YY1
0 hr
0.5 hr
1 hr
2 hr
YY1
65 KD
1 2 3 4
C) Blot
IP
Anti-YY1
Lysate
anti-myc
NRS
0 hr
0 hr
2 hr
2 hr
2 hr
2 hr
YY1
65 KD
1 2 3 4 5 6 7

Figure 1

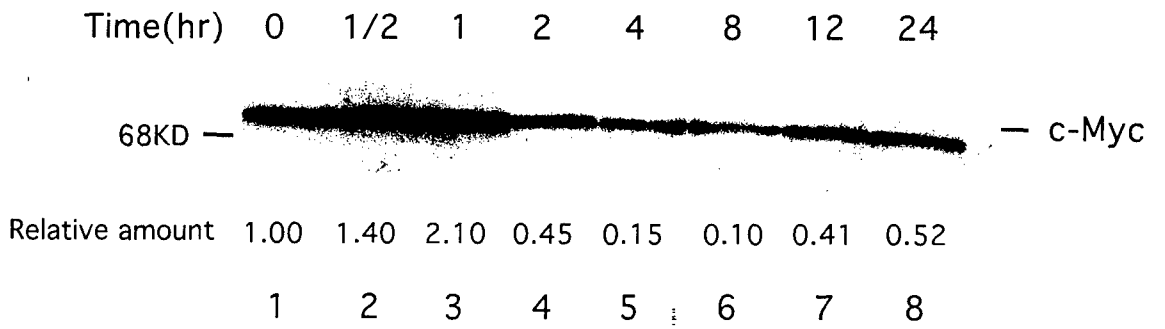



c-Myc binding domains on YY1. A. s35 labeled in vitro translated c-Myc protein were tested in binding assays with various YY1 N-terminal deletion GST fusion mutants. B. Binding assays of YY1 c-terminal deletion mutants.

Figure 2

A.


Time(hr) 0 1/2 1 2 4 8 12 24
68KD
c-Myc
Relative amount 1.00 1.40 2.10 0.45 0.15 0.10 0.41 0.52
1 2 3 4 5 6 7 8

B.

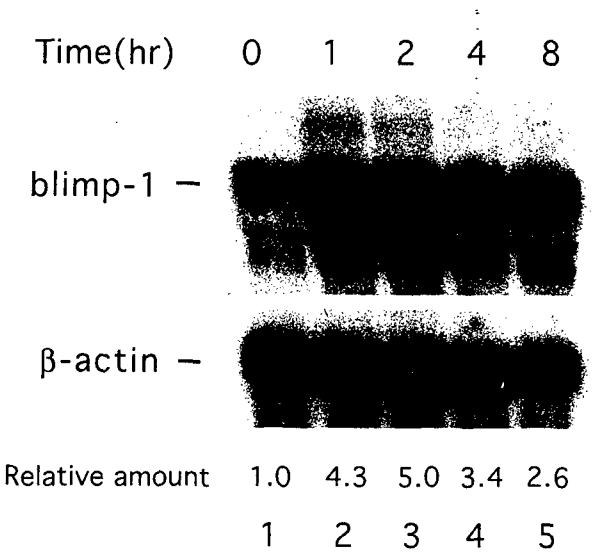

Time(hr) 0 1 2 4 8
blimp-1
β-actin
Relative amount 1.0 4.3 5.0 3.4 2.6
1 2 3 4 5

Figure 3

A

| Blimp AS | Blimp S | pSV2 | pSV2 c-Myc | G418 RESISTANT COLONIES |
|---|---|---|---|---|
| 5 | 0 | 10 | 0 | 1033 +/- 46 |
| 0 | 5 | 10 | 0 | 34 +/- 7 |
| 2 | 0 | 10 | 0 | 397 +/- 19 |
| 0 | 2 | 10 | 0 | 7 +/- 1 |
| 5 | 0 | 0 | 10 | 2011 +/- 153 |
| 0 | 5 | 0 | 10 | 347 +/- 73 |
| 2 | 0 | 0 | 10 | 599 +/- 24 |
| 0 | 2 | 0 | 10 | 340 +/- 11 |
| 2 | 0 | 0 | 20 | 684 +/- 19 |
| 0 | 2 | 0 | 20 | 446 +/- 30 |

B

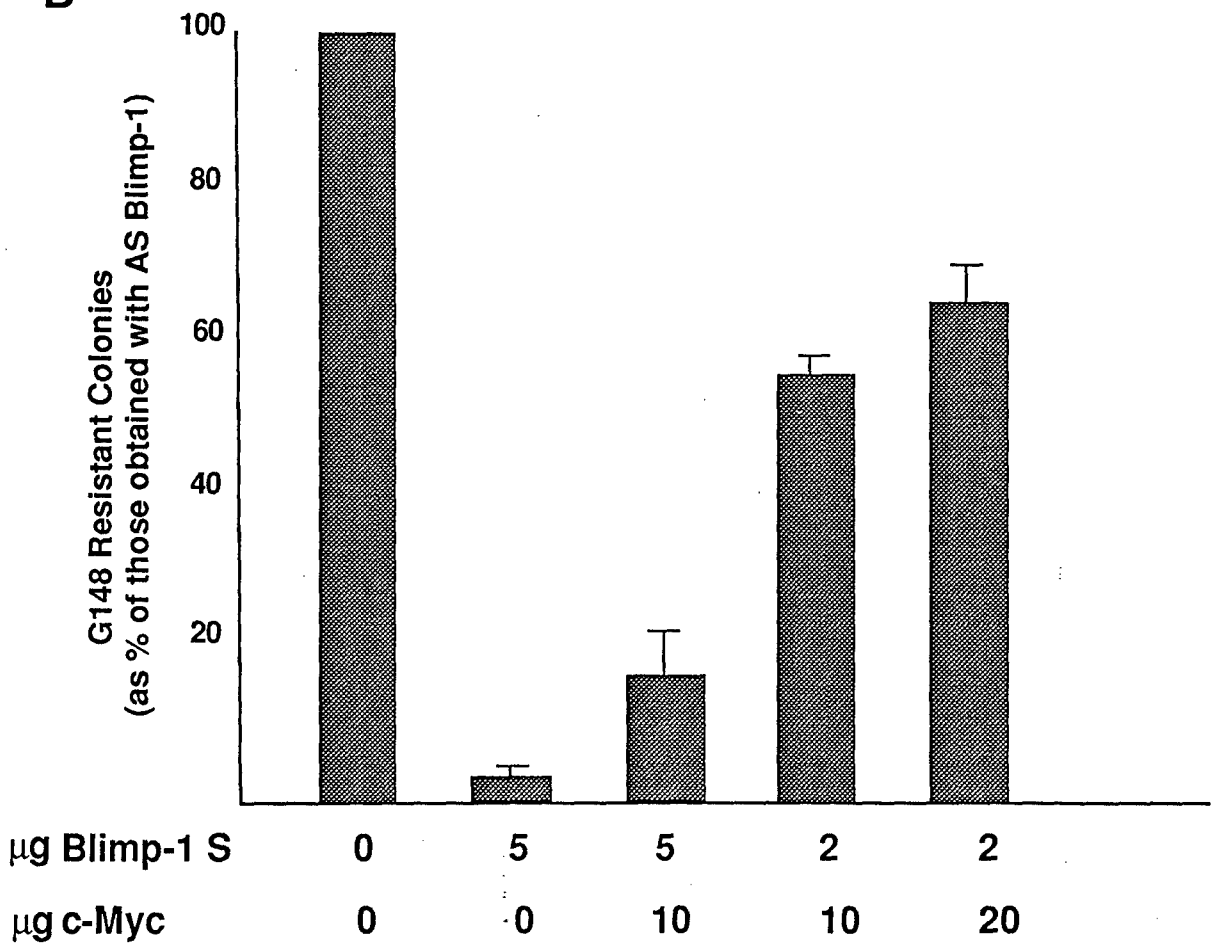
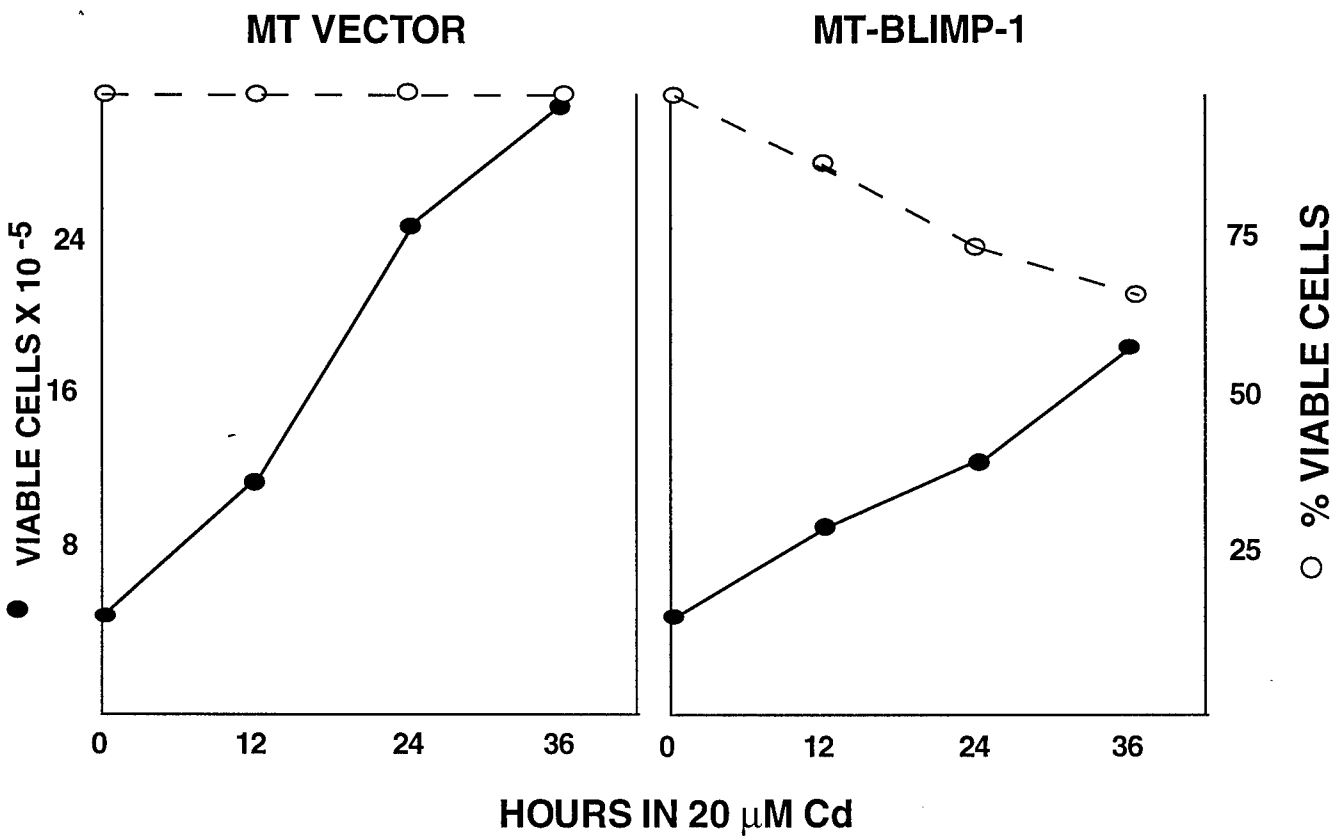



Figure 4


MT VECTOR
MT-BLIMP-1
VIABLE CELLS X 10 -5
24
16
8
75
50
25
% VIABLE CELLS
0
12
24
36
HOURS IN 20 μM Cd

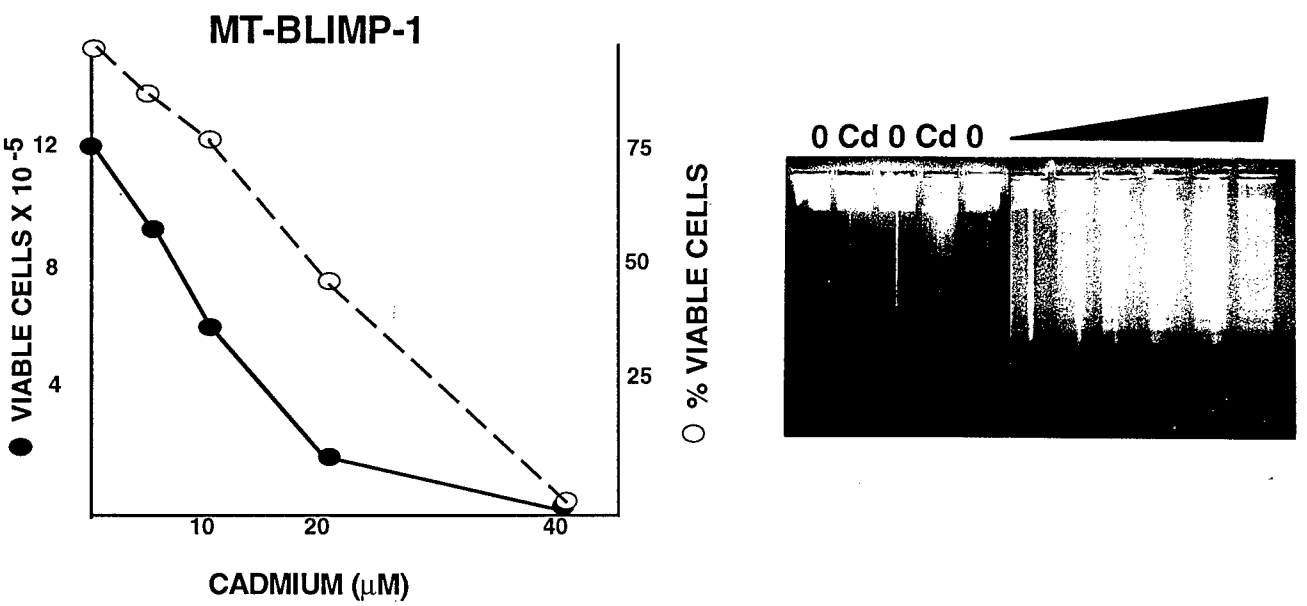

MT-BLIMP-1
VIABLE CELLS X 10 -5
12
8
4
75
50
25
% VIABLE CELLS
10
20
40
CADMIUM (μM)
0 Cd 0 Cd 0

Figure 5

Rec'd 1/11/2000

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 4 Jan 00

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCA, 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

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PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

94-J-4102 AD-B222 347
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